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# Suppression of Local Tissue Reactivity (Shwartzman Phenomenon) by Nitrogen Mustard, Benzol, and X-Ray Irradiation.

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When a filtrate of *Eberthella typhosa* is injected intradermally into a rabbit and 24 hours later injected intravenously, there develops a severe hemorrhagic necrotic reaction at the site of the intradermal injection within a period of 2-6 hours. Shwartzman[1] made his original observations on this reaction in 1927. It was soon found[2] that many unrelated bacteria were capable of producing the reaction, including the *Neisseria intracellularis* (Meningococcus), as well as non-bacterial substances, such as agar and starch. Although a certain small number of rabbits are naturally resistant to the reaction, a filtrate or endotoxin of a virulent meningococcus will consistently produce strong reactions in 90-100% of rabbits tested.

Although the histological reaction is basically one of vascular injury, opinions[2,3] differ as to the nature of the Shwartzman phenomenon. There is agreement, however, that it is a non-specific reaction which does not involve the known immunological mechanisms (agglutinins, precipitins, etc.). Because of the known depressive effect of Benzol, X-ray irradiation, and nitrogen mustards on the parenchymatous elements of the blood-forming organs[4-7] and on the reticuloendothelial system,[8-15] these agents were employed in an effort to throw light on the nature of the Shwartzman reaction.

*Methods and Materials.* The meningococcus filtrate and meningococcus endotoxin which were used throughout this study were prepared as follows:†

*Meningococcus filtrate*: Avirulent Type I strain of meningococcus was grown in 16 oz. flat-sided bottles for 24-30 hours on casein digest agar[16] enriched with cystein. The growth of each bottle was washed off with 10 cc of physiological saline in 0.4% phenol and centrifuged at approximately 3000 RPM for 20 minutes. The clear supernatant was passed twice through Berkefeld candle filters, adjusted to pH 7.8 and cultured for sterility.

Meningococcus endotoxin: The remaining sediment was resuspended in water (20 cc per culture bottle), adjusted to pH 8.2, incubated for 2 hours, placed in the icebox overnight, readjusted to pH 7.8, placed in a water bath at 60°C for 1 hour and 45 minutes, and cultured for sterility.

Fresh batches of the filtrate and the endotoxin were prepared approximately every 3

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† Kindly furnished by the laboratory of Dr. C. Phillip Miller.

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weeks and routine weekly cultures of both were sterile. The endotoxin was slightly more potent intravenously but both were found equally effective in eliciting severe hemorrhagic reactions in over 90% of the rabbits used. The control animals tested in any given group of rabbits received the same dose of the same substance by the same route as the treated animals of that group. The majority of the animals received the filtrate as the intradermal injection (*the skin preparatory factor*) and the endotoxin intravenously (*the intravenous reacting factor*).

*Animals*: Male albino rabbits weighing 2-3 kg were used. The hair on the abdomen was removed with electric clippers. No depilatories were used; no shaving was done.

*Intradermal injections*: Intradermal injections of 0.3 cc of the undiluted meningococcus filtrate or endotoxin were made in 3 different areas, the epigastrium, the right and left lower quadrants. The primary reactions resulting from the intradermal injections usually consisted of erythema and varying degrees of edema which would fade gradually over a 48-hour period if not followed in 24-48 hours by injection of the intravenous reacting factor.

*Intravenous injections*: From 20-26 hours after the intradermal injection, 2 cc of the undiluted meningococcus endotoxin per rabbit or 2 cc of the undiluted meningococcus filtrate per kg body weight was injected intravenously through the ear vein. A small percentage of the rabbits died within the first few hours after this injection from the inherent toxicity of the undiluted filtrate or endotoxin. Dilution was avoided to insure as high a percentage of severe reactions as possible.

*Results*: The effects of various agents were studied to determine their suppressive action on the Shwartzman phenomenon.

*Results in Untreated Control Animals.* Twenty-one normal untreated rabbits were used as controls. Of these, 19 developed severe hemorrhagic necrotic reactions measuring 3 - 6 cm in diameter at all 3 sites of the intradermal injections. One rabbit of the 21 was resistant and developed no reaction and the other rabbit died at the end of 4 hours post intravenous and had no skin reaction at the time of death. There was no consistent correlation noted between the intensity of the primary reaction from the intradermal injection itself and the severity of the hemorrhagic reaction after the injection of the intravenous reacting factor.

*Results in Treated Animals.* (1) The following reagents employed in near-lethal doses exerted no suppressive action on the Shwartzman reaction: benadryl, (10 mg IV *q* 3 h), urethane, (1 Gm bid subcut, for 1-14 days), penicillin G, (500,000 u and 1,000,000 u IM daily x 4 days), crude penicillin, (35 cc bid x 2 days), streptomycin, (0.5 Gm and 1.0 Gm daily IM x 4 days), British anti-lewisite, (50-100 mg bid subcut), sodium ascorbate, (75 mg daily subcut x 10 days), alpha tocopherol, (25 mg subcut daily x 10 days), protamine, (20 mg bid IV x 2 days), toluidine blue, (20 mg bid IV x 2 days), and mapharsen (13 mg/kg IV 1 inj.). Total thyroidectomy 3 weeks before the reaction and bleeding (30 cc daily x 2 days) also had no suppressive effect.

(2) *Following Total Body X-ray Irradiation.* When 13 rabbits were exposed once to 800 r total body irradiation 3-7 days before injection of the intravenous reacting factor there was almost complete suppression of the reaction in 7 of the rabbits and complete suppression of the reaction in the other 6 (Table I). One rabbit received a total body dose of only 300 r, 3 days before, and showed no suppression of the reaction. Two rabbits received 800 r total body irradiation apiece immediately before the injection of the intravenous reacting factor and these likewise showed no suppression of the reaction.

(3) *Following Benzol.* Benzol mixed with equal parts of sterile olive oil, was injected subcutaneously in doses of 1.0-2.0 cc of benzol per kg body weight (2.0-4.0 cc per kg of the benzol-olive oil solution) once daily for 7-13 days before injection of the intravenous reacting factor. The benzol was given daily until the rabbits showed outward signs of toxicity such as weight loss, refusal of food and lethargy. As noted in Table I, complete

TABLE I.
Effects of Benzol and X-ray Irradiation on the Shwartzman Phenomenon.

| Dosage schedule | | | Reactions following inj. I.V. reacting factor (4-6 hr) | | | |
|---|---|---|---|---|---|---|
| Amt. | Days before I.V. reacting factor* | No. of rabbits | None | ++ | ++++ | Deaths |
| Benzol—1.0-1.5 cc | 4 | 1 | | | | 1 |
| per kg daily | 7 | 4 | 2 | | | 2 |
| Subcut | 8 | 3 | 3 | | | |
| | 9 | 1 | 1 | | | |
| | 13 | 1 | 1 | | | |
| 2.0 cc per kg | 7 | 1 | | | | 1 |
| | 7 | 1 | 1 | | | |
| Totals | | 12 | 8 | 0 | 0 | 4 |
| X-ray total body irradiation | | | | | | |
| 800r | 0 | 2 | | | 2 | |
| 300r | 3 | 1 | | | 1 | |
| 800r | 3 | 2 | | 2 | | |
| 800r | 4 | 10 | 6 | 4 | | |
| 800r | 7 | 1 | | 1 | | |
| Totals | | 16 | 6 | 7 | 3 | |

None = no reaction.
++ = suppressed hemorrhagic reaction (1 cm).
++++ = severe hemorrhagic reaction (3-6 cm).
* = Meningococcus filtrate or endotoxin.

TABLE II.
Effect of Single Injection of Methyl-bis Nitrogen Mustard Intravenously on the Shwartzman Phenomenon.

| Dosage schedule | | | Reactions following inj. I.V. reacting factor (4-6 hr) | | | |
|---|---|---|---|---|---|---|
| Amt. | Days before I.V. reacting factor* | No. of rabbits | None | ++ | ++++ | Deaths |
| 2 mg/kg | 0 | 2 | | | 2 | |
| | 1 | 2 | | | 2 | |
| | 2 | 2 | 1 | 1 | | |
| | 3 | 9 | 9 | | | |
| | 4 | 4 | 3 | | | 1-1 hr |
| | 8 | 2 | 1 | 1 | | |
| | 11 | 2 | | 1 | 1 | |
| | 12 | 1 | | | 1 | |
| | 15 | 1 | | | 1 | |
| 1 mg/kg | 3 | 2 | 2 | | | |
| 0.5 mg/kg | 3 | 4 | | | 3 | 1-4 hr (no reac.) |
| Controls | | 21 | 1 | | 19 | 1-4 hr (no reac.) |

None = no reaction.
++ = suppressed hemorrhagic reaction (1 cm).
++++ = severe hemorrhagic reaction (3-6 cm).
* = Meningococcus filtrate or endotoxin.

suppression of the Shwartzman reaction was obtained in all 8 of the rabbits tested. Four rabbits, of the original 12 started with in this group, died during the period of benzol preparation.

(4) *Following Nitrogen Mustard.* Nitrogen mustard (methyl bis chlorethyl amine hydrochloride) was given in a single injection by the intravenous route with doses ranging from 0.5-2.0 mg/kg body weight, from 0-15 days before injection of the intravenous reacting factor. As can be seen from Table II,

there was no protection afforded when the mustard was given either at the same time as the intravenous reacting factor or when it was given on the day of the intradermal injection 20-26 hours before the intravenous reacting factor. When 2 mg/kg of nitrogen mustard was given 2 days before the intravenous reacting factor, there was partial suppression of the reaction in 1 rabbit and complete suppression in the other. When the nitrogen mustard was given in doses of 1-2 mg/kg 3-4 days before the injection of the intravenous reacting factor, the effects of the nitrogen mustard became more pronounced and there was complete suppression of the reaction in 14 of the 15 rabbits so treated, with the 15th rabbit dying during the first hour after injection of the intravenous reacting factor. When given 8-11 days before, the suppressive effect of the mustard was beginning to wane and in only one of 4 rabbits was there complete suppression, in 2 rabbits there was partial suppression and in 1 rabbit there was no suppression. When given the mustard 12 and 15 days before the injection of the intravenous reacting factor, the rabbits had completely regained their ability to react and, like the control rabbits, had severe hemorrhagic reactions. When a 0.5 mg/kg dose of mustard was used, no suppressive effect was noted.

*Discussion.* It is considered significant that the Shwartzman phenomenon of local tissue reactivity, even though a non-specific reaction, can be completely suppressed by nitrogen mustard, benzol, and X-ray, whose individual effects on blood-forming organs and the reticulo-endothelial system are so nearly identical. It is postulated that the mechanism of suppression by these agents is exerted through their specific but common suppressive action on the reticulo-endothelial system, primarily the vascular endothelium. These endothelial cells being rendered anergic are not able to react to the active principles in a way that otherwise would be self-destructive.

The Shwartzman phenomenon may then be interpreted as a local intracellular defensive but self-destructive reaction of the vascular endothelium to the bacterial active principles and not a reaction resulting from the direct toxic effects of these bacterial products on the cells. Thus, when the ability of the cell to react is interfered with or suppressed in a specific way, as exerted by nitrogen mustard, benzol, or X-ray, the integrity of the cell is maintained and a destructive process avoided.

It is suggested that this study provides an experimental basis for a new therapeutic concept in the treatment of diseases involving tissue and vascular reactivity to known and unknown toxins. This concept would be directed toward suppressing the ability of the vascular endothelium to react adversely to whatever circulating toxin might be the inciting agent. A group of these diseases would include active rheumatic fever, acute, subacute, and chronic disseminated lupus erythematosus, periarteritis nodosa, generalized vascular diseases due to hypersensitivity reactions to drugs, sera or vaccines, and probably dermatomyositis, rheumatoid arthritis, and acute and subacute glomerulonephritis. A recent case report by Osborne and associates[17] of the successful response of a case of chronic disseminated lupus erythematosus to nitrogen mustard would seem to provide confirmatory evidence in support of the therapeutic concept postulated above.

*Summary.* 1. The Shwartzman phenomenon was studied in rabbits using a meningococcus filtrate and meningococcus endotoxin. 2. The following agents were without suppressive effect on the Shwartzman phenomenon: benadryl, urethane, crude penicillin extract, penicillin G, streptomycin, mapharsen, BAL, vitamin C, alpha tocopherol, thyroidectomy, and partial exsanguination. 3. The reaction was completely suppressed by pretreatment of the rabbits with nitrogen mustard or benzol. It was also completely suppressed in some rabbits and partially in others by pretreatment with total body X-ray irradiation. 4. It is suggested that this study provides an experimental basis for a new therapeutic concept in the treatment of diseases involving tissue and vascular reactivity.

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